

We Claim:

1. An electronically addressable microarray with a permeation layer containing first chemical groups for attaching to the microarray biomolecules and/or second chemical groups, said first groups having the formula:



wherein,

P is a chemical moiety for binding to the microarray and/or for binding a moiety of said second chemical group;

X is a chemical bond or a linking chemical moiety; and

R is a chemical moiety for attaching, either covalently or non-covalently, a derivatized biomolecule, or for attaching covalently a moiety of said second chemical groups.

2. A microarray according to claim 1 wherein **P** is selected from the group consisting of, alkenyl moieties including but not limited to α,β ,unsaturated carbonyls vinyl, allyl and homoallyl groups; epoxide, a chemical bond, phenyl boronic acid, salicylic hydroxamic acid, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.

3. A microarray according to claim 1 wherein **R** is selected from the group consisting of a chemical bond, streptavidin, a portion of streptavidin, biotin, phenyl boronic acid, salicylic hydroxamic acid, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.

4. A microarray according to claim 1 wherein **R** is a chemical moiety that is activated prior to participating in a chemical reaction for binding either a derivatized biomolecule or a moiety of said second chemical group.

5. A microarray according to claim 4 wherein said **R** is activated by either an increase or decrease in pH of a solution overlying said **R**.
6. A microarray according to claim 5 wherein said pH change is provided by an electronically generated potential of an electrode of an electronically addressable microarray.
7. A microarray according to claim 1 wherein **P** is further bonded with said second chemical groups wherein said second chemical groups have the formula **P'-X'-R'**, further wherein said **P** is bonded to the **P'** moiety of at least one said second chemical group.
8. A microarray according to claim 7 wherein said second chemical groups form a polymer wherein a backbone of said polymer comprises **P'** moieties connected to one another and **-X'-R'** are connected to each **P'**.
9. A microarray according to claim 8 wherein **P'** equals **P**, **X'** equals **X**, and/or **R'** equals **R**.
10. A microarray according to claim 1 wherein **R** is bonded with said second chemical groups wherein said second chemical groups have the formula **P'-X'-R'**, further wherein said **R** is bonded to the **P'** moiety of at least one said second chemical group.
11. A microarray according to claim 10 wherein said second chemical groups form a polymer wherein a backbone of said polymer comprises **P'** moieties connected to one another and **-X'-R'** are connected to each **P'**.
12. A microarray according to claim 11 wherein **P'** equals **P**, **X'** equals **X**, and/or **R'** equals **R**.

13. A microarray according to claim 1 wherein **X** is selected from the group consisting of a chemical bond, an alkyl group of 1-10 carbon atoms, an alkenyl group of 2-10 carbon atoms, alkyl esters, ketones, amides, ethers, thioesters, amido groups, and carbonyls, and any combinations thereof.
14. An electronically addressable microarray with a permeation layer containing first chemical groups and second chemical groups, said first groups having the formula **P-X-R** and said second groups having the formula **P'-X'-R'** wherein,
- P** is a chemical moiety for binding to the microarray, each of said **P** moieties further connecting said first groups to a permeable polymer of said microarray and to at least one **P'** of said second groups, said **P'** moieties each further connecting at least one other **P'** moiety of said second groups to form a polymer of said second groups;
- X** and **X'** are linking chemical moieties selected from the group consisting of a chemical bond, an alkyl group of 1-10 carbon atoms, an alkenyl group of 2-10 carbon atoms, alkyl esters, ketones, ethers, amides, thioesters, amido groups, and carbonyls, and any combinations thereof; and
- R** and **R'** are chemical moieties for attaching, either covalently or non-covalently, a derivatized biomolecule.
15. A microarray according to claim 14 wherein **R** and **R'** are selected from the group consisting of streptavidin, a portion of streptavidin, biotin, PBA, SHA, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
16. A microarray according to claim 15 wherein **R** equals **R'**.
17. A microarray according to claim 14 wherein said **P** or **P'** and **R** or **R'** are chemical moieties that are activated prior to participating in a chemical reaction

which activation is either under the same or mutually exclusive conditions, wherein said chemical reaction is between any of combinations of **P** and **P'**, **R** and a derivatized biomolecule, and/or **P'** and **P'** of another second group.

5 18. A microarray according to claim 17 wherein said **P**, **R**, **P'** or **R'** are activated by either an increase or decrease in pH of a reaction solution overlying said microarray.

10 19. A microarray according to claim 18 wherein said pH change is provided by an electronically generated potential of an electrode of an electronically addressable microarray.

15 20. A microarray according to claim 14 wherein **P** is activated to bond to free **P'**-**X'-R'** monomers in a chemical reaction comprising polymerization of said monomers, wherein said **P** bonds to a reactive center in the **P'** moiety of at least one of said free monomers wherein each of said **P'** moieties bind to at least one other **P'** of another monomer.

20 21. An electronically addressable microarray containing a permeation layer with first chemical groups and second chemical groups, said first groups having the formula **P-X-R** and said second groups having the formula **P'-X'-R'** wherein,

25 **P** is a chemical moiety for direct binding to the microarray, each of said **P** moieties further connecting said first groups to a permeable polymer of said microarray, said **R** is a chemical moiety connecting to at least one **P'** of said second groups, said **P'** moieties each further connecting at least one other **P'** moiety of said second groups to form a polymer of said second groups;

30 **X** and **X'** are linking chemical moieties selected from the group consisting of a chemical bond, an alkyl group of 1-10 carbon atoms, an alkenyl group of 2-10 carbon atoms, alkyl esters, ketones, amides, ethers, thioesters, amido groups, and carbonyls, and combinations thereof; and

R' is a chemical moiety for attaching, either covalently or non-covalently, a derivatized biomolecule.

22. A microarray according to claim **21** wherein **R** and **R'** are selected from the group consisting of streptavidin, a portion of streptavidin, biotin, phenyl boronic acid, salicylic hydroxamic acid, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
23. A microarray according to claim **22** wherein **R** equals **R'**.
24. A microarray according to claim **21** wherein said **P** or **P'** and **R** or **R'** are chemical moieties that are activated prior to participating in a chemical reaction which activation is either under the same or mutually exclusive conditions, wherein said chemical reaction is between any of combinations of **P** and **P'**, **R** and **P'**, and **P'** and **P'** of another second group.
25. A microarray according to claim **24** wherein said **P**, **R**, **P'** or **R'** are activated by either an increase or decrease in pH of a solution overlying said microarray.
26. A microarray according to claim **25** wherein said pH change is provided by an electronically generated potential of an electrode of an electronically addressable microarray.
27. A microarray according to claim **21** wherein **R** is activated to bond to free **P'-X'-R'** monomers in a chemical reaction comprising polymerization of said monomers, wherein said **R** bonds to a reactive center in the **P'** moiety of at least one of said free monomers wherein each of said **P'** moieties bind to at least one other **P'** of another monomer.

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- 5 28. An electronically addressable microarray having a permeation layer having first chemical moieties attached to biomolecules and/or to polymerized monomer units comprising second chemical moieties, said second chemical moieties further attached to biomolecules, wherein said attachment of said biomolecules to said first chemical moieties or to said polymerized units has occurred following activation of at least one of said first and/or said second chemical moieties under acidic and/or basic pH conditions, said first chemical moieties have the formula **P-X-R** and said second chemical moieties having the formula **P'-X'-R'**, wherein said **P** comprises a chemical element requiring activation for attaching to said permeation layer and/or to a **P'** of said second chemical moiety, said **X** and **X'** comprise nonreactive chemical elements, and said **R** and **R'** comprise chemical elements requiring activation different from **P** and **P'** for attaching said biomolecules or to **P'** of another second chemical moiety.
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- 15 29. A microarray of claim **28** wherein said permeation layer comprises a polymer polymerized from the monomer group consisting of acrylamide, bisacrylamide, methacrylamide, *N*-alkyl acrylamides, functionalized ethylene glycol derivatives, *N*-vinyl pyrrolidinone, bis-cystamine, acrylates, methacrylates, and acrylonitriles where alkyl refers to a carbon chain.
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30. A microarray of claim **28** wherein said biomolecules are derivatized with a chemical moiety selected from the group consisting of vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
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31. A microarray of claim **28** wherein said **P** and **P'** are selected from the group consisting of alkenyl moieties, α,β unsaturated carbonyls, vinyl, allyl and homoallyl groups, acetal, thioester, disulfide, epoxides, alkyl ether, and carboxylic acid.
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32. A microarray of claim **28** wherein said **X** and **X'** are selected from the group consisting of a chemical bond, a carbon chain consisting of 1 to 10 carbons, ethers, polyethers, amides, and esters.
- 5 33. A microarray of claim **28** wherein said **R** and **R'** are selected from the group consisting of alkenyl moieties, α,β ,unsaturated carbonyls, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, thioester, disulfide, epoxide, and alkyl ether.
- 10 34. A microarray of claim **33** wherein said **R** equals **R'**.
35. An microarray of claim **28** wherein adjustment of pH has been initiated by lowering or raising the pH of the buffer and/or by biasing electrodes of said electronically addressable array with either positive or negative electronic potential.
- 15 36. A microarray according to claim **28** wherein each of said **P**, **P'**, **R**, and or **R'** is a thioester.
- 20 37. A microarray according to claim **28** wherein each said **P**, **P'**, **R**, and or **R'** is an acetal moiety.
38. A microarray according to claim **28** wherein said **R** is selected from the group consisting of amines, derivatized amines, -salicylhydroxamic acid, bromoacetamide, salicyl hydroxamic acid, maleimide, streptavidin, biotin, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
- 25 39. A microarray according to claim **35** wherein said electronic potential has been applied at a current density of between 50 nA/5000 μm^2 and 5 μA /5000 μm^2 at a
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site on the microarray intended for activation for a time period between 30 and 600 seconds.

40. A method of making a microarray of biomolecules on an electronically addressable microchip comprising:
- a. binding chemical groups having the formula P-X-R to a permeation layer of said microchip;
 - b. activating a first chemical moiety represented by R of said formula so that said first moiety will react with and attach to a biomolecule having a second chemical moiety that will attach to said first moiety; and
 - c. contacting said activated first moiety of (b) with said second moiety of said biomolecule;
- wherein P of said formula represents a chemical moiety for attaching said chemical group to said permeation layer, X is a nonreactive moiety, and R is a chemical moiety for attaching said biomolecule.
41. A method according to claim 40 wherein said P chemical moieties are selected from the group consisting of alkenyl moieties, α,β ,unsaturated carbonyls vinyl, allyl and homoallyl groups, acetal, epoxide, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
42. A method according to claim 40 wherein said R chemical moieties are thioesters that are activated to become reactive thiols.
43. A method according to claim 40 wherein said R chemical moieties are acetal monomers that are activated to become aldehydes.

44. A method according to claim 40 wherein said X chemical moieties are selected from the group consisting of a chemical bond, amides, carbon chains consisting of 1 to 10 carbons, esters, ethers and polyethers.
- 5 45. A method according to claim 40 wherein said R chemical moieties are selected from the group consisting of a biotin, streptavidin, phenyl boronic acid, salicylic hydroxamic acid, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
- 10 46. A method according to claim 40 wherein said second moiety of said biomolecules are selected from the group consisting of a biotin, streptavidin, phenyl boronic acid, salicylic hydroxamic acid, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
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47. A method of making a microarray of biomolecules on an electronically addressable microchip comprising:
- 20 a. binding a polymer of chemical groups having the formula P-X-R to a permeation layer of said microchip wherein P of each group is a first chemical moiety forming a polymeric backbone such that P of each group is attached to at least one other P moiety and one of said P is used to bind said polymer to said permeation layer; and
- 25 b. contacting second chemical moieties represented by R of said formula with biomolecules having a derivative moiety that will bond either covalently or noncovalently to said second chemical moieties; wherein, X is a nonreactive moiety.
- 30 48. A method according to claim 47 wherein said P chemical moieties are selected from the group consisting of alkenyl moieties, α,β ,unsaturated carbonyls vinyl,

allyl and homoallyl groups, acetal, epoxide, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.

- 5 49. A method according to claim 47 wherein said R chemical moieties are thioesters that are activated to become reactive thiols.
50. A method according to claim 47 wherein said R chemical moieties are acetal monomers that are activated to become aldehydes.
- 10 51. A method according to claim 47 wherein said X chemical moieties are selected from the group consisting of a chemical bond, amides, carbon chains consisting of 1 to 10 carbons, esters, ethers and polyethers.
- 15 52. A method according to claim 47 wherein said R chemical moieties are selected from the group consisting of a biotin, streptavidin, phenyl boronic acid, salicylic hydroxamic acid, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
- 20 53. A method according to claim 47 wherein said derivatized moiety of said biomolecules are selected from the group consisting of a biotin, streptavidin, phenyl boronic acid, salicylic hydroxamic acid, vinyl, allyl, homoallyl, acetal, ester, carboxylic acid, amide, halo-acetamide, thiol, phosphorothiolate monoester, thioester, disulfide, aldehyde, ketone, hydrazide, hydrazine, and amines.
- 25 54. A method of making a microarray of biomolecules on an electronically addressable microchip comprising grafting chemical moieties having the formula P-X-R onto a permeation layer of an electronically addressable microarray wherein said grafting is carried out in the steps comprising:
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- a) contacting said permeation layer with a grafting slurry comprising a polymerizable monomer having the formula P-X-R and an activatable initiator to initiate polymerization and grafting, said P of said monomer comprising chemical moieties wherein P can bind to said permeation layer or polymerize with P moieties of other said monomers, said R of said monomer comprising chemical moieties wherein R can bind either covalently or noncovalently to a biomolecule having a moiety that is sensitive for binding to said R, and said X is selected from the group consisting of a chemical bond amides, carbon chains consisting of 1 to 10 carbons, ethers and polyethers;
- b) activating said initiator to initiate polymerization of said monomers and grafting onto said permeation layer; and
- c) contacting biomolecules having a chemical moiety that will bind to said R moieties wherein said biomolecules become bonded to said R moieties.

55. A method according to claim 54 wherein said initiator is selected from the group consisting of AIBN, ammonium persulfate and TEMED, benzoyl peroxide, D 4265, cerium (IV) sulphate, α, β, γ or X-rays, electron beam irradiation or initiation by plasma selected from the group consisting of Argon, Nitrogen, and Oxygen.

56. A method according to claim 54 wherein said permeation layer is a hydrogel.

57. A method according to claim 56 wherein said hydrogel comprises polymerized acrylamide.

58. A method according to claim 54 wherein said polymerizable monomer is selected from the group consisting of alkenyl moieties, α, β , unsaturated carbonyls vinyl, allyl and homoallyl groups.

59. A method according to claim 54 wherein said R is a moiety that must be activated prior to binding with said biomolecules.
- 5 60. A method according to claim 54 wherein said permeation layer is a sol-gel.
61. A method of making a microarray of biomolecules on an electronically addressable microchip comprising grafting chemical moieties having the formula P-X-R onto a permeation layer of an electronically addressable microarray wherein said grafting is carried out in the steps comprising:
- 10 a) contacting said permeation layer with a grafting solution comprising a polymerizable monomer having the formula P-X-R and an initiator requiring activation to initiate polymerization and grafting, said P of said monomer comprising chemical moieties wherein P can bind to said permeation layer as well as polymerize with P moieties of other
- 15 said monomers, said R of said monomer comprising chemical moieties wherein R can bind either covalently or noncovalently to a biomolecule having a moiety that is sensitive for binding to said R, and said X is selected from the group consisting of a chemical bond amides, carbon chains consisting of 1 to 10 carbons, ethers and
- 20 polyethers; *a*
- b) activating said initiator to initiate polymerization of said monomers and grafting onto said permeation layer; and
- 25 c) contacting biomolecules having a chemical moiety that will bind to said R moieties wherein said biomolecules become bonded to said R moieties.
62. A method according to claim 61 wherein said permeation layer is a hydrogel.
- 30 63. A method according to claim 62 wherein said hydrogel comprises polymerized acrylamide.

64. A method according to claim 61 wherein said initiator is selected from the group consisting of AIBN, benzoyl peroxide, D 4265, cerium (IV) sulphate, α,β,γ or X-rays or initiation by plasma selected from the group consisting of Argon, Nitrogen, and Oxygen.
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65. A method according to claim 61 wherein said polymerizable monomer is selected from the group consisting of alkenyl moieties, α,β ,unsaturated carbonyls vinyl, allyl and homoallyl groups.
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66. A method according to claim 61 wherein said R is a moiety that must be activated prior to binding with said biomolecules.
- a*
- add B4*